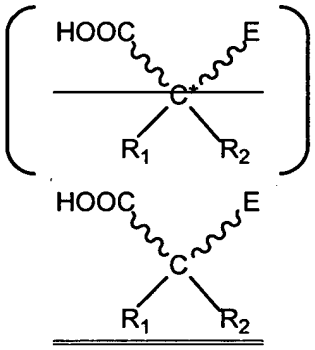
CLAIM AMENDMENTS

1. (Currently amended): A method for producing an enantiomerically pure α-substituted carboxylic acid, said method comprising ~~contacting an aldehyde or ketone with a cyanide containing compound and an ammonia containing compound or an ammonium salt or an amine, and~~ stereoselectively hydrolyzing reaction components ~~a resulting amino nitrile or cyanohydrin intermediate~~ with ~~a recombinantly generated nitrile or polypeptide having~~ a nitrilase ~~activity~~, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid,

wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2, or SEQ ID NO:4, or an enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4.

2. (Currently amended): The method according to claim 1, wherein said enantiomerically pure α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is -H or lower alkyl.

3. (Currently amended): The method according to ~~claim 2~~ claim 1, wherein said enantiomerically pure α-substituted carboxylic acid is an α-amino acid.

4. (Original): The method according to claim 3, wherein at least one of R_1 and R_2 is substituted or unsubstituted aryl.

5. (Currently amended): The method according to ~~claim 4~~ claim 3, wherein said enantiomerically pure α-amino acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

6. (Original): The method according to claim 3, wherein said enantiomerically pure α-amino acid bears a substituted or unsubstituted alkyl side chain.

7. (Original): The method according to claim 6, wherein said enantiomerically pure α-amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.

8. (Currently amended): The method according to ~~claim 2~~ claim 1, wherein said enantiomerically pure α-substituted carboxylic acid is an α-hydroxy acid.

9. (Canceled)

10. (Currently amended): The method according to ~~claim 10~~ claim 8, wherein said enantiomerically pure α-hydroxy acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

11. (Currently Amended): The method according to claim 47 ~~claim 1~~, wherein the cyanide is a metal cyanide or a gaseous cyanide.

12. (Original): The method according to claim 11, wherein the cyanide is an alkali cyanide.

13. (Original): The method according to claim 11, wherein the metal cyanide is sodium cyanide.

14. (Currently Amended): The method according to ~~claim 1~~ claim 47, wherein the ammonia salt has the formula $NH_2(R)_2^+X$, wherein each R is independently -H or lower alkyl, and X is a counter ion.

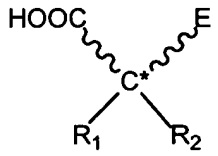
15. (Original): The method according to claim 14, wherein X is a halide.

16. (Original): The method according to claim 15, wherein the halide is Cl^-.

17. (Original): The method according to claim 16, wherein the ammonia salt is $NH_4^+Cl^-$.

18. (Withdrawn): An enantiomerically pure α-substituted carboxylic acid produced by a process comprising combining an aldehyde or ketone with a metal cyanide, ammonia or an ammonium salt, and a nitrilase, under conditions and for a time sufficient to produce the carboxylic acid.

19. (Withdrawn): The enantiomerically pure α-substituted carboxylic acid according to claim 18, having the structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is -H or lower alkyl.

20. (Withdrawn): The enantiomerically pure α-substituted carboxylic acid according to claim 19, wherein the carboxylic acid is an α-amino acid.

21. (Withdrawn): The enantiomerically pure α-substituted carboxylic acid according to claim 18, wherein the carboxylic acid is an α-hydroxy acid.

22. (Canceled)

23. (Currently amended): The method according to claim 1, wherein the nitrilase or an enzymatically active fragment thereof, is encoded by a nucleic acid sequence ~~as set forth in~~ consisting of SEQ ID NO: 1, or SEQ ID NO:3, or subsequences thereof encoding the enzymatically active ~~fragments~~ fragment of [[a]] the nitrilase.

24. (Currently Amended): ~~The method according to claim 1~~ A method for producing an enantiomerically pure α-substituted carboxylic acid, said method comprising stereoselectively hydrolyzing reaction components with a nitrilase, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid,

wherein the nitrilase has an amino acid sequence having at least 70% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, wherein the amino acid sequence encodes an enzyme that retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4~~, and has a nitrilase activity such that the nitrilase stereoselectively hydrolyzes an amino nitrile or cyanohydrin intermediate to form the enantiomerically pure α-substituted carboxylic acid~~.

25. (Withdrawn): A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity.

26. (Withdrawn): An isolated nucleic acid sequence encoding an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity, and fragments thereof that hybridize to the nucleic acid sequence.

27. (Withdrawn): An isolated nucleic acid sequence as set forth in SEQ ID NO:1.

28. (Withdrawn): An isolated nucleic acid sequence as set forth in SEQ ID NO:3.

29. (Withdrawn): A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2.

30. (Withdrawn): A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:4.

31. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

(a) providing an aldehyde or a ketone;

(b) providing a cyanide-containing compound;

(c) providing an ammonia-containing compound or a compound comprising an ammonium salt or an amine;

(d) providing a composition comprising a ~~recombinantly generated~~ nitrilase ~~or a polypeptide having a nitrilase activity~~, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2, or SEQ ID NO:4, or an enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4;

(e) contacting the aldehyde or ketone of step (a) with a cyanide-containing compound of ~~step (c)~~ step (b) and an ammonia-containing compound or a compound comprising an ammonium salt or an amine of ~~step (d)~~ step (c) such that an amino nitrile or a cyanohydrin intermediate is produced; and

(f) contacting the amino nitrile or cyanohydrin intermediate of step (e) with the composition of step (d) such that the nitrilase ~~or polypeptide~~ stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

32. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

(a) providing a composition comprising an amino nitrile or a cyanohydrin;

(b) providing a composition comprising a ~~recombinantly generated~~ nitrilase ~~or a polypeptide having a nitrilase activity~~, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2, or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4; and

(c) contacting the amino nitrile or cyanohydrin of step (a) with the composition of step (b) such that the nitrilase ~~or polypeptide having nitrilase activity~~ stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

33. (Currently amended): The method of claim 31 or 32, wherein the nitrilase ~~or polypeptide having nitrilase activity~~ stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an enantiomerically pure alpha-substituted carboxylic acid.

34. (Previously presented): The method of claim 31 or 32, wherein the alpha-substituted carboxylic acid is an alpha amino acid.

35. (Previously presented): The method of claim 31, wherein the cyanide-containing compound comprises a metal or a gaseous cyanide compound.

36. (Currently amended): A method for stereoselectively producing an alpha-amino acid, the method comprising

(a) providing an aldehyde or a ketone;

(b) providing a cyanide-containing compound and ammonia;

(c) providing a ~~recombinantly generated~~ nitrilase ~~or polypeptide having a nitrilase activity~~, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2, or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4;

(d) contacting the aldehyde or ketone of step (a) with the cyanide-containing compound and ammonia of step (b) such that an amino nitrile is produced; and

(e) contacting the amino nitrile of step (d) with the nitrilase ~~or polypeptide having nitrilase activity~~ of step (c) such that the nitrilase ~~or polypeptide~~ stereoselectively hydrolyzes the amino nitrile to produce an alpha-substituted amino acid.

37. (Previously presented): The method of claim 31, claim 32 or claim 36, wherein the reaction takes place in a single reaction vessel.

38. (Currently amended): The method of claim 24, wherein the nitrilase has an amino acid sequence having at least 75% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4.

39. (Currently amended): The method of claim 38, wherein the nitrilase has an amino acid sequence having at least 80% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4.

40. (Currently amended): The method of claim 39, wherein the nitrilase has an amino acid sequence having at least 85% sequence identity to an amino acid sequence ~~as set forth in~~

consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragments thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4.

41. (Currently amended): The method of claim 40, wherein the nitrilase has an amino acid sequence having at least 90% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4.

42. (Currently amended): The method of claim 41, wherein the nitrilase has an amino acid sequence having at least 95% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active ~~fragments~~ fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4.

43. (Previously presented): The method of claim 24, wherein the sequence identity is determined using a FASTA version 3.0t78 algorithm with default parameters.

44. (Currently amended): A method for stereoselectively producing an ~~enantiomerically pure~~ alpha-substituted carboxylic acid, said method comprising

contacting an aldehyde or ketone with a cyanide-comprising compound and an ammonia-comprising compound, an ammonium salt or an amine, and

hydrolyzing stereoselectively the resulting amino nitrile or cyanohydrin intermediate with a nitrilase, wherein the nitrilase hydrolyzes the reaction components to stereoselectively produce ~~enantiomerically pure~~ an alpha-substituted carboxylic acid and wherein the nitrilase has (i) an amino acid sequence having at least 70% sequence identity to an amino acid sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 70% sequence identity to an ~~amino acid~~ nucleic acid sequence ~~as set forth in~~ consisting of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes an enzyme that retains the same enzymatic activity as nucleic acid sequence from which it varies.

45. (New): The method of claim 1, wherein the reaction takes place in a single reaction vessel.

46. (New): The method of claim 24, wherein the reaction takes place in a single reaction vessel.

47. (New): The method of claim 1, wherein the reaction components are an aldehyde or ketone, a cyanide containing compound, and an ammonia-containing compound or ammonia salt or an amine.

48. (New): The method of claim 24, wherein the reaction components are an aldehyde or ketone, a cyanide containing compound, and an ammonia-containing compound or ammonia salt or an amine.

49. (New): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2, or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4; and

contacting reaction components with the composition such that the nitrilase stereoselectively hydrolyzes the reaction components to produce an alpha-substituted carboxylic acid.

50. (New): A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising hydrolyzing stereoselectively the reaction components with a nitrilase, wherein the nitrilase has (i) an amino acid sequence having at least 70% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 70% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes an enzyme that retains the same enzymatic activity as nucleic acid sequence from which it varies.